

Environmentally mediated trends in otolith composition of juvenile Atlantic cod (*Gadus morhua*)

**Ryan R.E. Stanley¹, Ian R. Bradbury^{1,2}, Claudio DiBacco³,
Paul V.R. Snelgrove¹, Simon R. Thorrold⁴, and Shaun Killen⁵**

¹ Ocean Sciences Center and Biology Department., Memorial University of Newfoundland, St. John's, NL A1C 5S7, P.O. Box 4200, Canada

² Department of Fisheries and Oceans, Northwest Atlantic Fisheries Centre, St. John's, NL, A1C 5X1, Canada

³ Department of Fisheries and Oceans, Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, NS, B2Y 4A2, Canada

⁴ Biology Department, MS #50, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts, 02543, USA.

⁵ Institute of Biodiversity, Animal Health & Comparative Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow G12 8QQ, United Kingdom

Corresponding author: rstanley@mun.ca 1 (709) 864-4469

Abstract:

We evaluated the influence of environmental exposure of juvenile Atlantic cod (*Gadus morhua*) to inform interpretations of natal origins and movement patterns using otolith geochemistry. Laboratory rearing experiments were conducted with a variety of temperature (~ 5, 8.5 and 12 °C) and salinity (~ 25, 28.5 and 32 PSU) combinations. We measured magnesium (Mg), manganese (Mn), strontium (Sr) and barium (Ba), expressed as a ratio to calcium (Ca), using laser ablation inductively coupled plasma mass spectrometry (ICP-MS), and stable carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotopes using isotope ratio monitoring mass spectrometry. Temperature and salinity significantly affected all elements and isotopes measured, with the exception of salinity on Mg:Ca. We detected significant interactions among temperature and salinity for Mn:Ca and Ba:Ca partition coefficients (ratio of otolith chemistry to water chemistry), with significant temperature effects only detected in the 32 and 28.5 PSU salinity treatments. Similarly, we detected a significant interaction between temperature and salinity in incorporation of $\delta^{13}\text{C}$, with a significant temperature effect except at intermediate salinity. These results support the contention that environmental mediation of otolith composition varies among species, thus limiting the ability of generalized models to infer life history patterns from chemistry. Our results provide essential baseline information detailing environmental influence on juvenile Atlantic cod otolith composition, punctuating the importance of laboratory validations to translate species-specific otolith composition when inferring *in situ* life histories and movements.

KEYWORDS: Otolith chemistry, temperature, salinity, *Gadus morhua*, elemental fingerprinting, isotopes

Introduction:

The application of otolith geochemistry to resolve natal habitats or to reconstruct life histories assumes otolith chemistry corresponds to local environmental conditions and ambient water chemistry, and that these conditions vary to impart a unique geochemical signal (Campana, 1999). Physiological and environmental factors both mediate assimilation of elements and isotopes into otoliths (e.g. Thorrold et al., 1997b; Elsdon and Gillanders, 2002). Prediction of where otolith composition differences might exist, or the reconstruction of fish movements and environmental histories, requires predictable relationships between environmental variables and otolith chemistry (Elsdon et al., 2008; Reis-Santos et al., 2013). Laboratory studies that have quantified specific relationships between select environmental factors and otolith composition (Hoff and Fuiman, 1995; Bath et al., 2000; Elsdon and Gillanders, 2002; Elsdon and Gillanders, 2003; Martin and Wuenschel, 2006; Miller, 2011; Reis-Santos et al., 2013) highlight a lack of generality across among different species (Gillanders, 2005; Reis-Santos et al., 2008; Barnes and Gillanders, 2013). For instance, Sr:Ca ratios in otoliths vary considerably among species in the absence of differences in dissolved Sr:Ca in ambient water (Campana, 1999), presumably resulting from inter-specific differences in metabolic traits (Kalish, 1991; Campana et al., 2000; Miller, 2011).

Ambient concentrations of elements and isotopes in seawater may also co-vary with temperature and salinity (Epstein and Mayeda, 1953; Elsdon and Gillanders, 2003; Reis-Santos et al., 2013, this study). Retrospective assignment of adults to nursery habitats or environmental conditions during the juvenile period has been accomplished through

geochemical analysis of material close to the otolith core, corresponding to the juvenile period (Thorrold et al., 2001; Gillanders, 2005; Farmer et al., 2013). Determining the effects of temperature and salinity on otolith elemental and isotopic incorporation will enhance our ability to predict where significant differences might occur and interpret life history movements (Elsdon and Gillanders, 2002). Although use of otolith signatures does not require explicit understanding of all details influencing incorporation (Campana et al., 2000), the significant influence of environmental factors adds complexity to the interpretation of geochemical concentrations in isolation of environment (Martin and Wuenschel, 2006).

Atlantic cod (*Gadus morhua*) is a demersal finfish species native to the North Atlantic, spanning from Greenland to Cape Hatteras, North Carolina to as far east as the Baltic Sea. Spawning occurs in both inshore and offshore environments, between late winter and early spring. Pelagic eggs develop into larvae that later metamorphose into benthic juveniles inhabiting offshore areas as well as shallow inshore regions, often associating with complex habitat (Laurel et al., 2003). The large latitudinal and depth range of Atlantic cod exposes juveniles to a gradient of temperatures and salinities (Dalley and Anderson, 1997), making them ideal candidates for studies on environmental effects on otolith elemental incorporation. Atlantic cod have received considerable research attention, in particular efforts to understand the structure of remaining fragmented populations in the Northwest Atlantic. The pelagic egg and larval phase imparts a large dispersal potential, mediated by spawn timing and ambient conditions (Bradbury et al., 2000; Stanley et al., 2013). Genetic (Ruzzante et al., 2000), tagging (Rose et al., 2011), and otolith chemistry techniques (Campana et al., 1999; Jamieson et al., 2004; D'Avignon and Rose, 2013) have successfully delineated spawning aggregation

spatial structure, but fall short of providing information on critical juvenile habitat and potential links to adult populations.

The use of otolith chemistry for retrospective evaluation of environmental history, or to aid in delineating nursery habitat, requires a baseline understanding of the influence of environment on otolith composition. Controlled laboratory experiments provide a vehicle to evaluate relationships between otolith composition and the environment. Interactive effects of temperature and salinity (e.g. Miller, 2011) necessitate orthogonally designed experiments to fully evaluate environmental drivers on otolith chemistry (Elsdon and Gillanders, 2002). The objective of this study was to evaluate the influence of temperature and salinity on the biogenic incorporation of trace elements in the otoliths of juvenile Atlantic cod, a commercially important species that spans a wide range of these environmental parameters. Specifically, we measured concentrations of magnesium (Mg), manganese (Mn), strontium (Sr) and barium (Ba), expressed as a ratio to calcium (Ca), and stable isotopes of carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$); past studies show some form of environmental mediation in incorporation of all of these elements. Specifically, we utilized controlled laboratory conditions to determine the relative and interactive effects of temperature and salinity on elemental and isotopic concentrations of juvenile cod otoliths.

Methods:

Experimental Design

Juvenile Atlantic cod were obtained from a common broodstock provided by Atlantic Genome Canada and the Dr. Joe Brown Aquatic Research Building (JBARB) at the Ocean Sciences Centre (OSC), Memorial University of Newfoundland (MUN). Newly hatched fish

were reared at ambient conditions (~ 11 °C and 32 PSU) for up to 137 days before they were distributed among the experimental treatments. Forty fish (~ 4 cm standard length) were moved to each experimental aquaria (40 L) and acclimated to desired temperature treatment by adjusting cold room temperatures by 2 °C every 2 days, in order to slowly acclimate fish to the new thermal regime from the common start point of 11 °C. When desired temperature treatments were achieved, salinities were manipulated by 2 PSU every two days, until the new salinity regimes were reached from the common start point of 32 PSU. Once acclimation and treatment levels were achieved, fish were immersed in seawater treated with Alizarin Red-S (ARS) (600 Mg·l⁻¹) at a pH adjusted to 7.0, for 24 hours. Staining in Alizarin Red induces a fluorescent tag that clearly indicated the start of the experiment and the relevant otolith material to sample (Figure 1; Beckman and Schulz, 1996). A standardized finfish pelletized diet (EWOS ®) was fed to all fish to minimize any dietary effects of food on otolith chemistry (Walther and Thorrold, 2006; Reis-Santos et al., 2013). Through the experiment fish were fed to saturation *ad-libitum* three times daily and maintained at a 12-hour light cycle.

Three experimental temperature treatments of approximately 5, 8.5 and 12 °C, hereafter referred to as low, medium, and high temperatures, were maintained in three separate cold rooms at the OSC. For each temperature treatment we used three separate salinity treatments of 25, 28.5 and 32 PSU, hereafter referred to as low, medium, and high salinities. Each temperature by salinity treatment was replicated three times for a total of 27 experimental aquaria, and nine unique combinations. Salinities were achieved through dilution of filtered seawater from the JBARB (32 ±0.25 PSU) using non-chlorinated well water from the Marine Institute of Memorial University. Set salinity treatments were pre-mixed and stored in each cold room for 24 hours prior to use in tanks to avoid the need for any

acclimation during daily water exchanges. Partial water exchanges (50%) were performed daily along with siphoning any excess food, waste, or dead fish. Temperature and salinity were checked for consistency daily using a YSI-55 probe. In addition, dissolved oxygen, pH, and ammonia levels were monitored every second day with the YSI probe and ammonia test meters. To minimize ammonia levels, each aquarium was equipped with a Bio-Wheel filter and ammonia filter pads.

Otolith preparation and geochemical analysis

Fish were exposed to experimental treatments for a total of 90 days (14 July – 12 October 2007). Upon completion of the experiment, we removed the fish and recorded standard length. Both sagittal and lapillal otoliths were removed, cleaned with ultrapure water, air dried, and stored in acid-washed glass vials. We later mounted otoliths on glass microscope slides and polished them using 0.3 μm lapping film. Mounted otoliths were cleaned again with a nylon brush, triple rinsed in ultrapure water and sonified for 2 min. We then air dried otoliths in a laminar flow hood, prior to transfer to clean petri dishes, and transport to the Woods Hole Oceanographic Institution Plasma Mass Spectrometry Facility for analysis. One lapillus was randomly selected for laser ablation and one sagitta was randomly selected for $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analysis.

We measured otolith elemental composition using a Thermo Finnigan *Element2* inductively coupled plasma mass spectrometer (ICP-MS) coupled with an ArF excimer 193-nm laser ablation system. We chose to quantify five elements (^{25}Mg , ^{43}Ca , ^{55}Mn , ^{88}Sr , and ^{138}Ba), that were consistently higher in the otolith samples than in HNO_3 blanks during preliminary analyses and have shown potential for environmentally mediated otolith

incorporation. We lasered along a line parallel to the otolith edge within a common growth band and outside of the Alizarin mark ($\sim 500 \mu\text{m}$). Laser repetition rate was set at 5 Hz for all analyses, with a scan speed of $5 \mu\text{m s}^{-1}$. We used a certified reference material (CRM) consisting of powdered otoliths (Yoshinaga et al., 2000), dissolved in 2% ultrapure HNO_3 (SeaStar[®]) and diluted to a Ca concentration of $40 \mu\text{g}\cdot\text{g}^{-1}$, to correct for instrument bias and drift following Thorrold and Swearer (2009). External precision was estimated by analyzing a second otolith CRM (Sturgeon et al., 2005), also dissolved in 2% HNO_3 (SeaStar[®]) and diluted to a Ca concentration of $40 \mu\text{g}\cdot\text{g}^{-1}$, periodically throughout the laser analyses ($n = 55$). Analytical accuracy was determined from the concentrations of Japanese Otolith and NRC standards, averaged across all samples. We found that accuracy was exceeded 98% for all otolith constituents measured, including isotopic ratios described later. We calculated limits of detection (LOD) as 5 times the mean intensity of blanks (2% HNO_3), run every 10 assays for each otolith constituent. Any measurements below the respective LOD were excluded from analysis. We conducted all statistical analyses using otolith elemental compositions converted to molar values and standardized to calcium concentrations ($\text{Me}:\text{Ca}_{\text{otolith}}$). Estimates of external precision based on the relative standard deviation values of the second CRM were 4.2% for Mg, 17.9% for Mn, 0.40% for Sr, and 1.4% for Ba.

We measured $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in sagittal otoliths of each individual by milling otoliths with a MicroMill sampler (New Wave Research) or a handheld dental drill under a stereomicroscope until we obtained 50 to 200 μg of near edge material. Stable isotopes were measured using an isotope ratio monitoring mass spectrometer according to methods outlined by Ostermann and Curry (2000). We report values of both isotopes relative to Vienna Pee Dee

Belemnite (VPDB ‰). Long-term precision estimates based on the use of NBS-19 were ± 0.07 for $\delta^{18}\text{O}$ and ± 0.03 for $\delta^{13}\text{C}$ (Ostermann and Curry, 2000).

Water analyses

We used ICP-MS to measure the chemical composition of water samples taken from each experimental treatment ($n=27$) at the mid-point of the experiment (day 32). Water intake for the OSC and JBARB comes from approximately 5 m depth with a very stable salinity (± 0.25 PSU, yearly variance) and temperature (± 1 °C, weekly variance) (Danny Boyce, JBARB facilities manager, Memorial University of Newfoundland, St. John's Canada, A1C 6N3, personal communication). Low variance in water condition indicates a relatively stable intake water mass, with no expected major changes in ambient water chemistry during the course of the experiment. Because water for all treatments was obtained from a common filtered and stable source, we assume samples collected represent the average conditions experienced over the course of the experiment aside from any temperature or salinity effects. For each treatment, a 50-ml aliquot of water was sampled, acidified with ultrapure nitric acid, and subsequently analyzed for elemental composition. Samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista Pro). Methods for analysis were adapted from EPA Method 200.7 (ICP-OES). Elemental calibration standards were prepared from 1000 mg l⁻¹ reference solutions and original stocks are NIST-Traceable. Precision estimates are not provided because only one water sample from each treatment was analyzed. Elemental concentrations were standardized to and expressed as element to calcium ratios ($\text{Me}:\text{Ca}_{\text{water}}$), which were used in all subsequent statistical analyses.

Growth

Upon experiment completion, we recorded standard length for each fish. Because all fish came from the fertilization event, and were harvested at the same time, we used standard length of the fish as measure of somatic growth. We also measured otolith deposition using the Alizarin stain as a marker for the beginning of the experiment. Otolith length was defined as the length of a transect radiating out from the beginning of the alizarin stain to the outer edge of the otolith. In addition, we measured pre-experimental growth from the otolith core to the beginning of the alizarin stain (Figure 1). Pre-experimental otolith length provides an index scoring the relative size of individuals placed into experimental treatments.

Statistical Analysis

Elemental ($\text{Me:Ca}_{\text{otolith}}$ & $\text{Me:Ca}_{\text{water}}$) and isotope data were inspected for normality using Q-Q plots and clear outliers (i.e., more than two times the standard deviation from the mean of a given metric) were removed. In total, less than 3% of observations met these exclusion criteria. Partition coefficients provide a complementary metric to compare otolith incorporation rates across experimental treatments accounting for both $\text{Me:Ca}_{\text{otolith}}$ and $\text{Me:Ca}_{\text{water}}$ (Reis-Santos et al., 2013), and are especially useful for comparisons among species and studies (Martin and Wuenschel, 2006). We calculated partition coefficients (D_{me}) for each element by dividing the measured otolith calcium ratio ($\text{Me:Ca}_{\text{otolith}}$) by the observed water ratio ($\text{Me:Ca}_{\text{water}}$) of the treatment (Morse and Bender, 1990). The majority of field studies only provide baseline information based on observed otolith composition because the ambient water chemistry is often missing and therefore partition coefficients cannot be calculated (but see, Thorrold et al., 1998). For this reason, we present analyses of otolith composition ($\text{Me:Ca}_{\text{otolith}}$) and partition coefficients (D_{me}) in tandem.

Statistical analyses were conducted in R-stats version 3.1.0 (R Development Core Team, 2014). Differences in growth, water chemistry, otolith chemistry, and partition coefficients, as a function of experimental treatment, were analysed as general mixed-effects models (LME) using the “*lme4*” package in R. Response variables (i.e. D_{me}) were \log_{10} transformed and treated as continuous variables in response to our categorical fixed treatments, temperature and salinity. To account for growth effects, we constructed a temperature by salinity growth model, using the same mixed-effects model outlined previously, and employed the residuals as an index of growth variability which accounts for correlations with environment. Growth residuals were incorporated into LME models as continuous covariates. Replicate tanks, within each treatment, were treated as a nested random categorical factor for all models. We did not report the non-significant random tank effect because it was unimportant to the statistical question posed in the analysis (Bolker et al., 2009). Each element and isotope was analyzed individually, thus creating 6 separate statistical tests. To control for family-wise error rates and multiple comparisons, the significance of each test was adjusted using a *Benjamini-Hochberg* (Benjamini and Hochberg, 1995) correction and compared to a significance level set at $\alpha = 0.05$. In instances where we detected significant differences, post-hoc pairwise tests using the ‘*multcomp*’ package in R determined which treatments differed.

Random effects

For all mixed-effects models we found no significant replicate tank effect. All p-values exceeded 1.2 except otolith $\delta^{18}\text{C}$ which approximated 0.08.

Ethics

All experimental procedures and fish handling were approved and conducted in accordance to the Canadian Council of Animal Care Guidelines and Memorial University of Newfoundland animal utilization protocols.

Results:

Rearing conditions

Experimental conditions were consistent throughout the trials for low (5.0 ± 0.2 °C and 25.1 ± 0.1 PSU; Mean \pm SD), intermediate (8.6 ± 0.3 °C and 28.5 ± 0.2 PSU; Mean \pm SD), and high (12.2 ± 0.3 °C and 31.8 ± 0.2 PSU; Mean \pm SD) treatment levels (Figure 2). All water was sourced from a common intake and filtration system, but variation in ambient water chemistry occurred as a function of experimental conditions (Table 1). Salinity significantly affected Me:Ca_{water} ratios (Table 2), however, we detected no temperature, temperature-salinity interactions, or tank replicate effects in Me:Ca_{water}. Mg:Ca and Sr:Ca_{water} were positively correlated and Mn:Ca and Ba:Ca_{water} were negatively correlated with treatment salinity (Figure 3).

Otolith Chemistry

Temperature significantly influenced elemental-calcium (Me:Ca_{Otolith}) and isotope ratios (Tables 2 & 3 respectively; Figure 4). Salinity significantly influenced on all elements and isotopes tested, with the exception of magnesium (Mg:Ca_{Otolith}). We detected no interactive effects for any elemental ratio or for either stable isotope variables. Both Mg:Ca and Mn:Ca were significantly higher at the warmest temperatures. Mg:Ca and Sr:Ca ratios increased with salinity, whereas Mn:Ca and Ba:Ca ratios decreased (Figure 4), corresponding to trends observed in Me:Ca_{water} (Figure 3). Similarly, temperature and salinity significantly affected

isotope ratios. We did not find significant interactive effects of temperature and salinity on $\delta^{18}\text{O}$ ratios, but did find a significant interaction on ratios of $\delta^{13}\text{C}$ (Table 3). Both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ associated positively with salinity across all temperature treatments. $\delta^{13}\text{C}$ values were significantly lower at the low temperatures compared to intermediate and high temperature treatments, except at the intermediate salinity treatment (thus the significant interaction between temperature and salinity). Conversely, we observed significantly higher $\delta^{18}\text{O}$ in the low temperature treatment compared to intermediate and high temperatures (Figure 4).

Partition Coefficients

Ranges of elemental partition coefficients, D_{Me} (Table 1), were similar to those reported in previous studies (Martin et al., 2004; Martin and Thorrold, 2005; Martin and Wuenschel, 2006; Miller, 2011; Reis-Santos et al., 2013). Estimates of D_{Me} for all elements showed a significant influence of temperature and a similar effect for salinity for all elements except D_{Mg} . We found significant interactive effects between temperature and salinity for D_{Mn} and D_{Ba} (Table 2, Figure 5). D_{Mg} showed a weakly significant positive association with temperature only. Temperature significantly influenced D_{Mn} only at the highest salinity treatment. D_{Mn} associated positively with salinity for all temperature treatments, especially at the highest temperature. Salinity influenced D_{Sr} coefficients, particularly at low temperatures, where the intermediate salinity treatment had a significantly lower mean D_{Sr} value than low or high salinity treatments. D_{Sr} associated negatively with temperature, with the largest mean D_{Sr} in the lowest temperature treatments (Figure 5). For D_{Ba} , we observed a temperature effect only in the high salinity treatment where coefficients were significantly greater than the low temperature treatment. Barium partition coefficients tracked positively with salinity in all

temperature treatments with highest coefficients associated with the highest salinity. We observed temperature effects in D_{Ba} only in the high salinity treatment.

Growth

We found no significant difference among experimental treatments in pre-experimental otolith length, confirming that all individuals were similar in size before experimental rearing (ANOVA: $F=0.99$ $p=0.38$, $F=0.44$ $p=0.64$, for temperature and salinity respectively). Somatic and otolith growth were highly correlated (Pearson $r=0.58$, $p<0.0001$) and temperature affected both variables significantly, with lowest growth in the coldest temperature treatment (Figure 6). Collectively, otoliths grew proportionately faster than the fish themselves, resulting in proportionally smaller otoliths in faster growing fish (log linear model: slope = 1.4 $t=12.7$, $p<0.00001$). Salinity did not significantly affect growth rate singularly, but we did observe a weakly significant interaction between temperature and salinity for somatic growth (Table 4). In low temperature treatments, growth was significantly higher at intermediate salinities.

Mixed-effects models revealed significant relationships for $Me:Ca$ and D_{Me} with Mg , Mn , and Sr , as well as ratios of $\delta^{13}C$, with treatment growth residuals (Tables 2 and 3). All estimated slopes were positive except for D_{Sr} and $Sr:Ca$, and we observed no significant relationships between growth residuals and $Ba:Ca$, D_{Ba} and $\delta^{18}O$. These trends mirror directionality and significance of correlative relationships among otolith constituents and growth across treatments (Supplementary Table 1).

Discussion:

Otolith geochemistry provides potentially powerful methodology for identifying natal habitats and inferring environmental histories of juvenile Atlantic cod, potentially allowing individuals in adult populations to be linked to specific juvenile or natal nursery habitat. Reliable prediction and evaluation of fish environmental history requires, however, an understanding of how the environment mediates incorporation of ambient elements and isotopes into fish otoliths. Broadly speaking, the influence of temperature and salinity on otolith elemental composition remains ambiguous, with laboratory studies (e.g. Bath et al., 2000; Elsdon and Gillanders, 2002; Elsdon and Gillanders, 2003; Miller, 2011; Reis-Santos et al., 2013) highlighting strong species-specific relationships (Reis-Santos et al., 2008; Melancon et al., 2009), likely attributable to variable physiology (Miller, 2011). Indeed, there are few examples of consistent relationships between otolith chemistry and environment among fish species (Campana, 1999). Laboratory validations thus provides an important and necessary step in evaluating biotic and abiotic influences on otolith incorporation rates (Kalish, 1991; Miller, 2011) for specific elements and isotopes prior to field application (Reis-Santos et al., 2013). Our experiment represents an important contribution towards understanding the application of otolith microchemistry to environmental reconstructions of juvenile Atlantic cod (*Gadus morhua*) and similar coastal marine fishes.

Our experiments demonstrate significant influences of both temperature and salinity on otolith composition of juvenile Atlantic cod (*Gadus morhua*). Gradients of temperature, salinity, and ambient chemical conditions (Me:Ca_{water}), driven by variation in upwelling and mixing of marine and freshwater inputs (Reis-Santos et al., 2013) often characterize coastal nursery habitats in which fish reside or move. Our experiment included a range of

temperatures experienced by juvenile Atlantic cod in the Newfoundland inshore environment (Dalley and Anderson, 1997; Craig and Colbourne, 2004), therefore providing a realistic template on which to test the relationship between environment and otolith composition.

Otolith chemistry

Both temperature and salinity significantly affected almost all of the $\text{Me}:\text{Ca}_{\text{Otolith}}$ and D_{Me} variables. Only $\text{Mg}:\text{Ca}_{\text{Otolith}}$ and D_{Mg} were influenced by temperature alone. Previous laboratory experiments evaluating the effects of environment on elemental incorporation found significant effects of both temperature and salinity both with (Secor et al., 1995; Elsdon and Gillanders, 2002; Barnes and Gillanders, 2013) and without (e.g. Martin and Thorrold, 2005; Martin and Wuenschel, 2006) an interaction. Significant interactions between temperature and salinity brings into question generalities drawn from assessments where uncontrolled factors such as temperature or salinity in coastal environments may add environmental heterogeneity and bias otolith signals (Elsdon and Gillanders, 2002).

Temperature and salinity both significantly influenced $\text{Sr}:\text{Ca}$ ratios and partition coefficients. We observed highest $\text{Sr}:\text{Ca}_{\text{Otolith}}$ values in the highest salinity treatments (~32 PSU). The result was expected as $\text{Sr}:\text{Ca}_{\text{Otolith}}$ commonly occurs in proportion to ambient availability (Farrell and Campana, 1996; Campana et al., 1999) and measurements of water chemistry showed the highest $\text{Sr}:\text{Ca}_{\text{Water}}$ ratios at the highest salinity across all temperature treatments. By calculating partition coefficients, we were able to examine the effect of temperature and salinity on $\text{Sr}:\text{Ca}_{\text{Otolith}}$ after accounting for differences in $\text{Sr}:\text{Ca}_{\text{Water}}$. While several studies found a positive effect of temperature on D_{Sr} (e.g. Bath et al., 2000; Martin et al., 2004), we found the opposite, with highest D_{Sr} values in the low temperature treatment,

similar to trends reported for larval Pacific, *Gadus macrocephalus* (DiMaria et al., 2010), and Atlantic cod (Townsend et al., 1995). At low temperatures fish apparently have a reduced ability to discriminate Sr incorporation into the otolith (Townsend et al., 1995), which mirrors our finding of higher Sr:Ca and D_{Sr} at the lowest temperature treatment. We found a dome-shaped effect of salinity on Sr partition coefficients, with lowest D_{Sr} values at intermediate salinities. Martin et al. (2004) also noted a significant effect of salinity (and hence dissolved Sr concentrations in the ambient water) on D_{Sr} and suggested that mutual inhibition of Ca and Sr ions across intestinal membranes may have generated this result. Our results are more complicated, as this inhibition would need to be complex and non-linear to generate the relation between salinity and D_{Sr} that we observed. Correspondingly Brown and Severin (2009) reviewed published otolith work and noted that marine species in general often exhibit equivalent or greater variation in Sr:Ca_{Otolith} ratios relative to diadromous and freshwater species, despite less variable ambient concentrations; these results suggest an equivocal contribution of Sr:Ca_{Water} levels to Sr:Ca_{Otolith}. Strontium is typically used as a chemical tracer of salinity, often defining transitions between freshwater and marine habitats (e.g. Bradbury et al., 2008). Studies that successfully developed otolith strontium and salinity relationships often utilized a larger gradient than used in our study (e.g. Martin and Wuenschel, 2006), and potentially spanned a greater range than that used as coastal juvenile cod habitat. The use of otolith strontium as an environmental tracer in Atlantic cod appears tenuous in isolation, though in combination with other elements might provide a contextual relationship to environment and a tracer for ambient Sr:Ca chemistry.

Barium incorporation correlated positively with temperature and negatively with salinity. Ba:Ca_{Otolith} ratios were significantly lower at the lowest temperature treatment with

decreasing levels at increasing salinity, as seen in previous studies with black bream (Webb et al., 2012). As expected, $Ba:Ca_{\text{Water}}$ did not differ significantly across temperature treatments, but did decrease significantly with salinity. Controlling for ambient water chemistry a different pattern emerges, with a significant temperature by salinity interaction influence on D_{Ba} . We observed higher D_{Ba} coefficients and detected temperature effects only at the highest salinity. Previous studies reported interactive influences of temperature and salinity on otolith $Ba:Ca$ ratios in Chinook salmon (Miller, 2011) and European seabass (Reis-Santos et al., 2013). Changes in $Ba:Ca_{\text{otolith}}$ and D_{Ba} patterns may be a product of water chemistry. Indeed, manipulations of ambient barium uniformly showed a clear influence on $Ba:Ca_{\text{otolith}}$ ratios (Miller, 2009; Collingsworth et al., 2010). Facilitation between Ba and Sr ions may also explain our observations. Previous studies highlighted elevated incorporation of Ba with increased Sr ratios (de Vries et al., 2005). Though our study did not manipulate Sr concentrations directly, we did observe the higher dissolved Sr concentrations, and lowest dissolved Ba concentrations, in the highest salinity treatment. A complementary hypothesis to Sr facilitation proposes that increased ambient water concentrations of Ba may inhibit incorporation of Ba into otoliths, based on the fact that Ba approaches proportional incorporation with the ambient water at low concentrations (Bath et al., 2000; de Vries et al., 2005).

Magnesium may offer promise as temperature proxy for juvenile Atlantic cod because only temperature significantly affected incorporation, measured by both $Mg:Ca$ and D_{Mg} . Increasing temperature led to increasing $Mg:Ca$ ratios in otoliths, both pooled across and within salinity treatments. This result confirms trends reported by Barnes and Gillanders (2013) and Elsdon and Gillanders (2002), who also found a significant positive relationship

between Mg:Ca and temperature but no effect of salinity. Mg:Ca is believed to be primarily under biological control (Martin and Thorrold, 2005), with otolith Mg concentration regulated by physiological fractionation between blood and endolymphatic fluids surrounding fish otoliths (Melancon et al., 2009). Temperature-mediated control of Mg:Ca_{otolith} ratios observed in our study and others may represent a product of thermal influence on the fractionation of Mg from blood to endolymphatic fluids (Barnes and Gillanders, 2013). Responses of otolith Mg:Ca ratios to temperature vary across taxa, with reports of non-significant (Martin and Thorrold, 2005; Martin and Wuenschel, 2006) and negative effects of temperature (Fowler et al., 1995). Variation in physiological response to temperature and its impact on fractionation likely contributes to among-species differences (Barnes and Gillanders, 2013).

Temperature and salinity significantly increased otolith manganese incorporation. Both Mn:Ca_{Otolith} and D_{Mn} were positively associated with temperature, with highest values in the warmest treatment pooled across salinity treatments. The influence of salinity was more varied with significantly lower Mn:Ca_{Otolith} and higher D_{Mn} ratios at higher salinities only when the temperature exceeded 5 °C. Considering partition coefficients, we found a significant temperature and salinity interaction on D_{Mn}, similar to previous work with juvenile spot, *Leiostomus xanthurus* (Martin and Thorrold, 2005). Temperature had a significant positive effect on D_{Mn} in high salinity treatments. As in Martin and Thorrold (2005), we found greater differentiation in D_{Mn} across salinity treatments at the warmest temperatures. Differences between Me:Ca and D_{Me} are often attributed to water chemistry variation across treatments (Martin and Wuenschel, 2006), and indeed we observed a significant negative association between Mn:Ca_{Water} and salinity as previously documented (Martin and Thorrold, 2005). However, past studies have not reliably linked ambient ratios of Mn (Mn:Ca_{Water}) to otolith

chemistry (Elsdon and Gillanders, 2003; Collingsworth et al., 2010). The signal-to-noise ratio of Mn is high relative to other elements, given sensitivity to changes in water chemistry and low otolith concentrations. Nonetheless, our observations of inverted trends with $\text{Mn:Ca}_{\text{Otolith}}$ and D_{Mn} potentially indicate limitation, however, without mediation of ambient conditions we cannot fully partition the influence of variable physiology and ambient availability on Mn incorporation. Despite a lack of consensus on the mechanisms underlying concentrations in fish otoliths, Mn has proven useful in discrimination analyses (Thorrold et al., 1998; Reis-Santos et al., 2008; D'Avignon and Rose, 2013). For Atlantic cod, Mn could potentially be used as a useful tracer for temperature; however, reliable reconstructions would be contingent upon some prior knowledge of salinity based on our results.

Stable C and O isotope chemistry offers a useful tool for reconstructing environmental histories of aquatic organisms because both isotope systems vary geographically in coastal and ocean waters (Thorrold et al., 1997a). Carbon isotopes ($\delta^{13}\text{C}$) in otoliths reflect a mixture of ambient dissolved inorganic carbon (DIC) and dietary carbon (Kalish, 1991; Schwarcz et al., 1998) sources in a ratio that is likely mediated by physiology (Thorrold et al., 1997a). Reported otolith ratios between ambient DIC and metabolic carbon vary from approximately 20% (Weidman and Millner, 2000) to 30% (Hoie et al., 2004) for Atlantic cod. We found a non-linear effect of temperature on $\delta^{13}\text{C}$ values, with decreases between intermediate and high temperature treatments as expected (Weidman and Millner, 2000) but with the lowest $\delta^{13}\text{C}$ values found at the coolest temperatures ($\sim 5^\circ\text{C}$). We held juvenile cod in the low temperature treatment at the bottom of their expected thermal range (Brander, 1995). Correspondingly, somatic and otolith growth were significantly reduced in the low temperature treatments. The result is, nonetheless, similar to that of Hoie et al. (2003) who observed higher $\delta^{13}\text{C}$ values

than low growth rate fish in larval and early juvenile Atlantic cod (<21 mm) with higher growth rates and presumably higher metabolism. Like Hoie et al. (2003), our observations cannot be attributed to diet, as we did not vary feeding among treatments, and all cultures were fed to saturation. Previous studies reported behavioural differences both in respiration rate (McConnaughey et al., 1997) and swimming alterations of metabolic activity (Bjornsson, 1993; Hoie et al., 2003) that correlated negatively with $\delta^{13}\text{C}$. We lacked a direct measure of either index but would not expect either behaviour to increase at lower temperatures.

Considering only the intermediate and high temperature treatments, where we observed no significant difference in size, $\delta^{13}\text{C}$ declined on average $-0.14\text{‰}/^{\circ}\text{C}$ ($p=0.001$, $r^2=0.37$), similar to rates derived from seasonal otolith records of wild caught Atlantic cod $\sim -0.16\text{‰}/^{\circ}\text{C}$ (Weidman and Millner, 2000). This rate is similar to that previously reported for bearded rock cod (Kalish, 1991), Atlantic croaker (Thorrold et al., 1997a), as well as various species of foraminifera and mollusks (Grossman and Ku, 1986) (-0.18 , -0.22 , -0.11 , and $-0.13\text{‰}/^{\circ}\text{C}$, respectively).

Otolith $\delta^{18}\text{O}$ decreased with temperature, confirming patterns previously reported for Atlantic cod (Hoie et al., 2004) and other biogenic aragonites (Grossman and Ku, 1986). Past studies documented negative associations of otolith $\delta^{18}\text{O}$ and temperature for a variety of species, and demonstrated the reliability of Atlantic cod as an indicator of temperature to within 1°C (Weidman and Millner, 2000) when adequately constraining the $\delta^{18}\text{O}$ of the ambient water.

Despite evidence linking salinity to variable isotope incorporation in fish otoliths (e.g. Schwarcz et al., 1998; Elsdon and Gillanders, 2002), no previous studies tested salinity effects

on otolith isotope concentrations in cod. We found a significant positive association between otolith isotopes and salinity. The positive association between salinity and $\delta^{18}\text{O}$ confirms previous studies documenting proportional otolith incorporation with ambient conditions (Thorrold et al., 1997a) and a positive relationship between water $\delta^{18}\text{O}$ and salinity (Gao, 2002).

Relationships with growth

Somatic and otolith growth rate can influence incorporation of elements and isotopes in fish otoliths (Hoie et al., 2003; Martin and Thorrold, 2005). Fish with faster growth rates generally have proportionally smaller otoliths (Worthington et al., 1995). In particular, past work found a negative correlation between otolith size and growth rate in juvenile Atlantic cod (Otterlei et al., 2002); our study shows that otoliths grow proportionally faster (~ 1.4) than fish length, confirming this trend. Diet and changes in physiology often associated with growth rate and size, influence carbon in particular (Hoie et al., 2003) as our data also showed. Somatic growth rate might be more important for larval and juvenile fishes where more variable growth is expected (Martin and Wuenschel, 2006). Our experiment revealed strong correlations between somatic growth and temperature (Bolland, 2008), which precluded a full evaluation of the combined effects of somatic growth with environmental condition resulting from limited variation individual growth within a treatment. A three-way temperature, salinity and feeding (growth) experiment similar to the nested design employed by Hoie et al. (2003) would most clearly elucidate the effect of variable growth rate on otolith incorporation (Martin and Wuenschel, 2006).

Though growth was not controlled orthogonally to environment in our experiment, we nonetheless observed variation in growth within treatments. Residuals from a temperature by

salinity growth model offer a tool to evaluate the effect of growth while accounting for environmental correlates. Our study found significant positive relationships between growth and otolith magnesium and manganese (Me:Ca and D_{Me}), similar to observations of D_{Mn} in Atlantic cod (Limburg et al., 2011) and other groundfish species (Limburg et al., 2014). Strontium (Sr:Ca and D_{Sr}) and growth were significantly and negatively related, as reported in juvenile striped bass (*Morone saxatilis*) (Secor et al., 1995). Incorporation of barium (Ba:Ca and D_{Ba}) was growth independent, as reported in adult Atlantic cod (Thorisson et al. (2011) and juvenile spot croaker (*Leiostomus xanthurus*) (Bath et al., 2000). Similarly, we observed an overall negative but non-significant relationship between oxygen ($\delta^{18}O$) incorporation and growth, suggesting growth independent incorporation, mirroring patterns previously reported for juvenile Atlantic cod (Hoie et al. (2003). Carbon ($\delta^{13}C$) ratios and growth were significantly and positively related, echoing similar trends in previous studies (Thorrold et al., 1997a). At low temperatures, growth was highest at intermediate salinities, where we also observed the highest $\delta^{13}C$ values. This relationship with growth likely drove the significant interaction between temperature and salinity on $\delta^{13}C$ incorporation. As with $\delta^{18}O$, the positive association between $\delta^{13}C$ and growth mirrors patterns observed for temperature and juvenile Atlantic cod (Hoie and Folkvord, 2006). Indeed all observed relationships between growth and otolith chemistry mirror temperature patterns. Collectively our results support the assertion that variation in physiology and otolith deposition associated with growth influence trace element incorporation (Secor et al., 1995), further highlighting the need for experimental studies that nest growth within temperature.

Conclusion

Ultimately, otolith geochemical composition provides a template to discern environmental histories of juvenile Atlantic cod, potentially providing novel information about this critical period. Analyses of otolith composition provides one method to assign juvenile residency in Atlantic cod, offering a feasible alternative to tagging studies that are logistically difficult or impossible in larval and juvenile fishes given their small size and relatively high mortalities (Thorisson et al., 2011). Spatial and temporal differences in otolith chemistry among geographic regions may reflect ambient water chemistry and environmental condition, noting the modest effect that diet alone has on otolith incorporation for most fish (Hoff and Fuiman, 1995; Milton and Chenery, 2001; Walther and Thorrold, 2006; Webb et al., 2012; Woodcock et al., 2012), with some notable acceptations (Buckel et al., 2004). The wide range of environmental conditions experienced by Atlantic cod in nearshore nursery habitat necessitates a comprehensive understanding of temperature and salinity effects on otolith chemistry. Moreover, these complex environments demand a more diverse suite of otolith trace elements and isotopes to ensure successful reconstruction. Results from our study provide a comprehensive assessment of temperature and salinity effects on a substantial suite of both elemental and isotopic constituents in juvenile Atlantic cod otoliths. Temperature and salinity both significantly affected all elements and isotopes we measured except Mg, which was apparently incorporated into otoliths independent of salinity. Collectively our results highlight the potential utility of otolith geochemistry to identify change and reconstruct environmental life histories of Atlantic cod, but they also highlight key considerations and limitations of the application when considering environmental variables in isolation.

Supplementary material

Additional supplementary material is available at ICES Journal of Marine Science online and the online version of this manuscript. Supplementary data is comprised of a table with the full temperature by salinity partitioned correlations between otolith constituents and fish growth, both otolith and somatic.

Acknowledgements

The authors thank the many individuals who assisted laboratory experiments. Special thanks go to Brenda Oake, Mary Ryan, Krista Boland, Jennifer Fitzgerald, Scott Birdwhistell, and Jerzy Bluztajn, without whom the experiment could not have been completed. Research funding and support was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) Strategic Grant on Connectivity in Marine Fishes. We are grateful to Atlantic Genome Canada for providing the juvenile cod used in this study. R. Stanley was supported by an NSERC Postgraduate Scholarship and a Research and Development Corporation of Newfoundland (RDC) student fellowship. All experiments were conducted in accordance with Canadian and institutional animal care guidelines.

Tables:

Table 1. Means, standard deviations (sd) and ranges for each element and isotope *Gadus morhua* otolith constituent measured across all treatments. Trace elemental data is standardized as a ratio of calcium; units for elements are per mol Ca, Isotopic ratios of $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ are expressed in ‰ relative to VPDB, and partition coefficients are ratios of otolith chemistry to ambient water chemistry.

Parameter	Mean	Sd	Range	Units	n
<i>Me:Ca_{Water}</i>					
Mg	5.31	0.57	(5.06-5.53)	Mol	24
Mn	5.99	2.60	(2.26-9.50)	μmol	24
Sr	9.40	0.23	(8.87-9.74)	mmol	24
Ba	22.98	14.8	(5.23-44.25)	μmol	24
<i>Me:Ca_{Otolith}</i>					
Mg	20.19	11.15	(7.27-61.7)	μmol	278
Mn	2.62	1.05	(1.02-6.07)	μmol	273
Sr	3.08	0.76	(1.68-5.05)	mmol	273
Ba	2.49	0.79	(1.32-4.54)	μmol	273
$\delta^{18}\text{O}$	-3.68	0.79	(-5.3--1.87)	‰	116
$\delta^{13}\text{C}$	-0.57	0.98	(-2.57-1.46)	‰	116
<i>D_{Me}</i>					
Mg	3.81	2.1	(1.43-11.3)	*10 ⁻⁶	269
Mn	0.50	0.27	(0.129-1.72)	-	267
Sr	0.28	0.08	(0.18-0.53)	-	265
Ba	0.18	0.14	(0.033-0.73)	-	269

Table 2: Results of linear mixed effects models comparing water chemistry (Md:Ca_{Water}), otolith chemistry (Me:Ca_{Otolith}) and elemental partition coefficients (D_{Me}) as a function of temperature (T) and salinity (S) with replicate tank controlled as a random variable. Residuals from the temperature x salinity growth relationship were evoked as a continuous covariate. To control for multiple comparisons p-values are adjusted according to the *Benjamini-Hochberg* transformation.

	Source of Variation	df	Mg		Mn		Sr		Ba	
			F	p	F	p	F	p	F	p
Me:Ca _{Water}	T	2	1.8	0.354	5.4	0.042	0.5	0.879	0.3	0.904
	S	2	238.0	<0.0001	194.5	<0.0001	324.4	<0.0001	1233.0	<0.0001
	T:S	4	0.2	1.000	1.9	0.337	0.6	0.879	0.2	0.924
Me:Ca _{Otolith}	T	2	6.8	0.003	14.2	<0.0001	27.7	<0.0001	27.5	<0.0001
	S	2	1.3	0.343	14.4	<0.0001	6.2	0.004	17.2	<0.0001
	G	4	4.7	0.046	15.0	<0.0001	31.6	<0.0001	0.3	0.728
	T:S	9	0.1	0.984	0.6	0.799	0.1	0.984	1.8	0.192
D _{Me}	T	2	7.2	0.002	34.8	<0.0001	28.0	<0.0001	25.9	<0.0001
	S	2	0.3	0.888	117.6	<0.0001	4.2	0.029	742.4	<0.0001
	G	4	4.7	0.049	15.0	<0.0001	31.6	<0.0001	0.3	0.606
	T:S	9	0.1	0.974	2.7	0.041	0.1	0.974	2.6	0.046

Table 3: Results of linear mixed effects models comparing otolith isotopes Carbon and Oxygen ratios among temperature (T) and salinity (S) treatments with replicate tank controlled as a random variable. Residuals from the temperature x salinity growth relationship were evoked as a continuous covariate. To control for multiple comparisons p-values are adjusted according to the *Benjamini-Hochberg* transformation.

	Source of Variation	df	$\delta^{18}\text{O}$		$\delta^{13}\text{C}$	
			F	p	F	p
Me:Ca _{Otolith}	T	2	61.39	<0.0001	43.72	<0.0001
	S	2	23.23	<0.0001	21.70	<0.0001
	G	4	0.02	0.914	0.95	0.030
	T:S	9	1.72	0.208	4.67	<0.0001

Table 4: Results of linear mixed effects models comparing somatic and otolith growth as a function of temperature (T) and salinity (T). Tank replicate treated as a random factor.

Source of variation	df	Somatic		Otolith	
		F	p	F	p
T	2	102.4	<0.0001	34.0	<0.0001
S	2	1.7	0.23	2.9	0.06
S x T	4	5.3	0.001	1.7	0.16

Figures

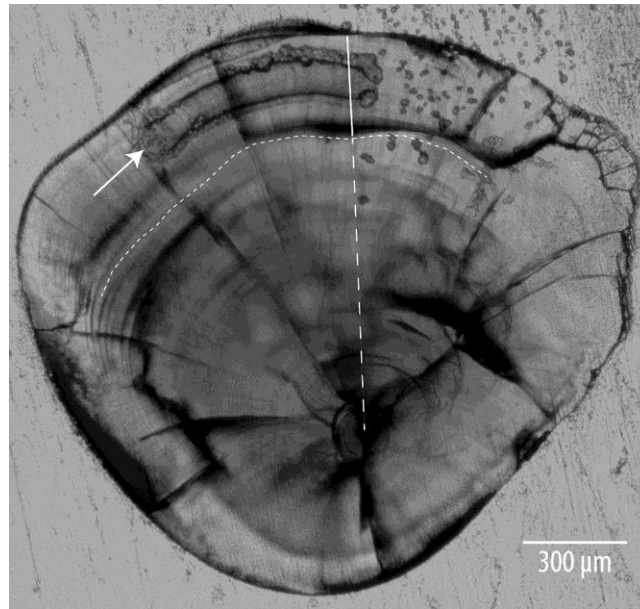


Figure 1: Polished juvenile *Gadus morhua* sagittal otolith showing laser raster (arrow), otolith growth (solid line), pre-experimental growth (long dashed line) and alizarin tag (short dashed line) denoting beginning of the experiment.

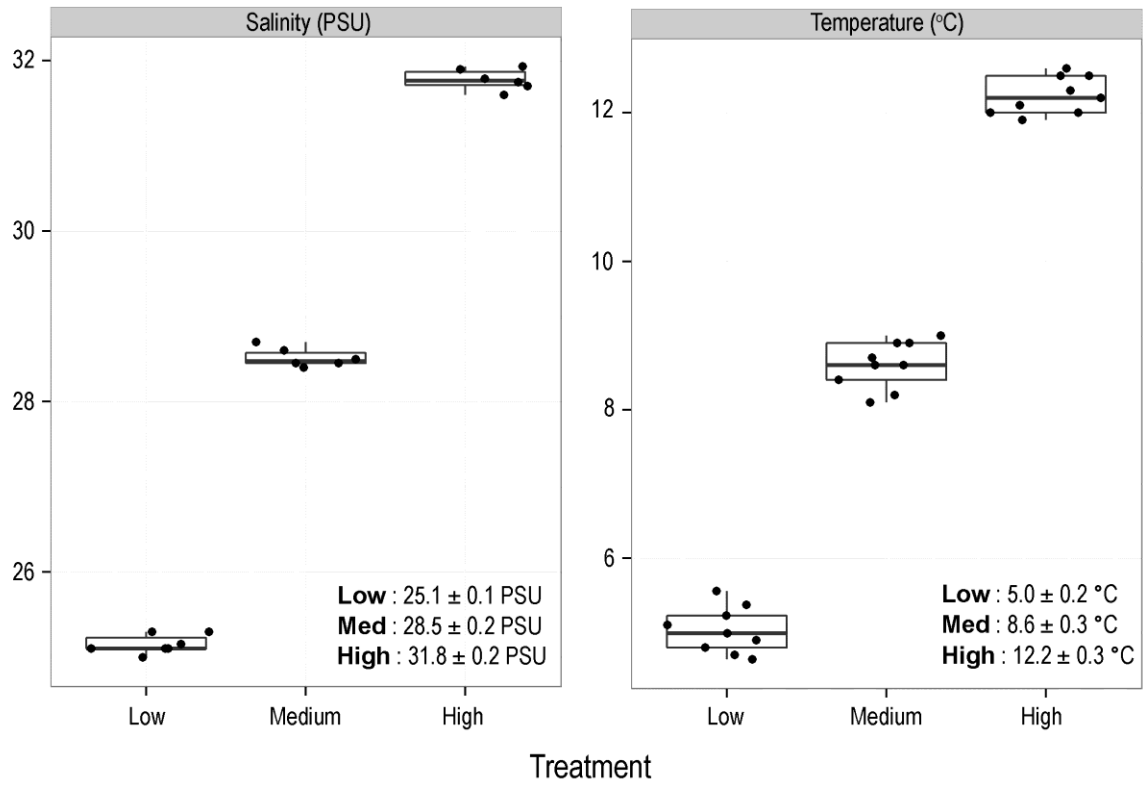


Figure 2: Summary of experimental treatments conditions. Means values are reported ± 1 standard deviation.

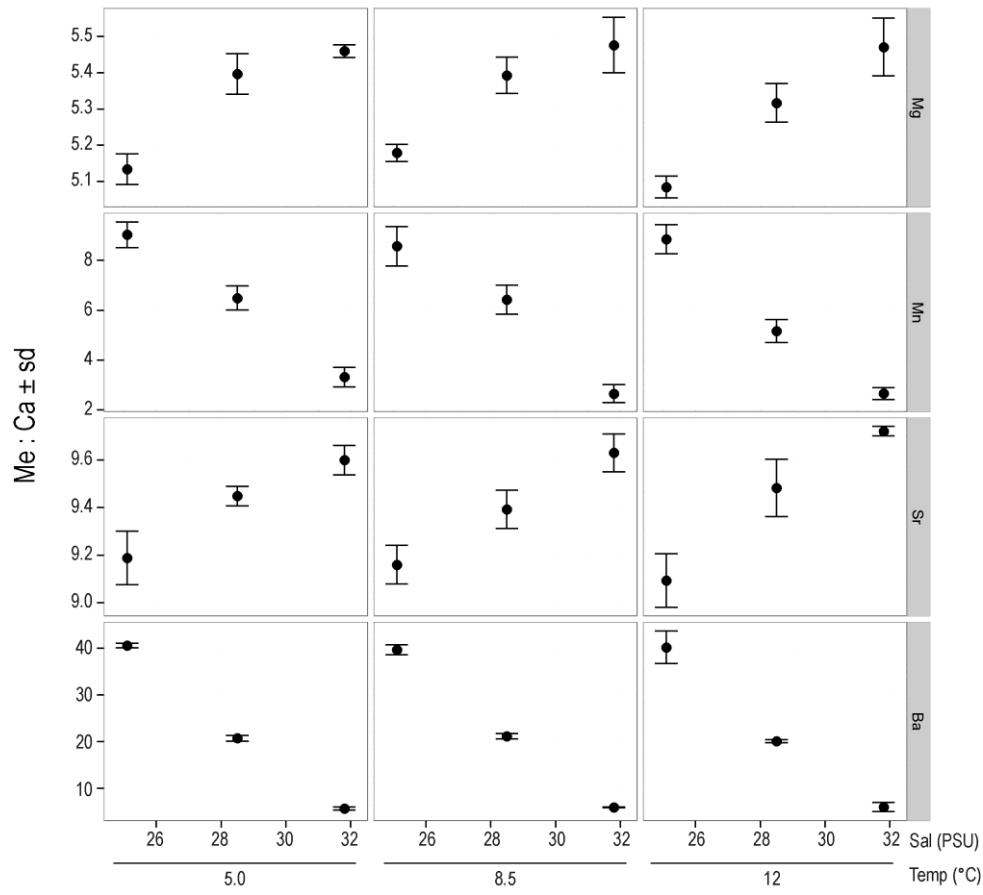


Figure 3: Mean ratios of Me:Ca_{water} among temperature and salinity experimental treatments. Mg:Ca values are in mol·mol⁻¹, Mn:Ca and Ba:Ca are μmol·mol⁻¹, and Sr:Ca is mmol·mol⁻¹.

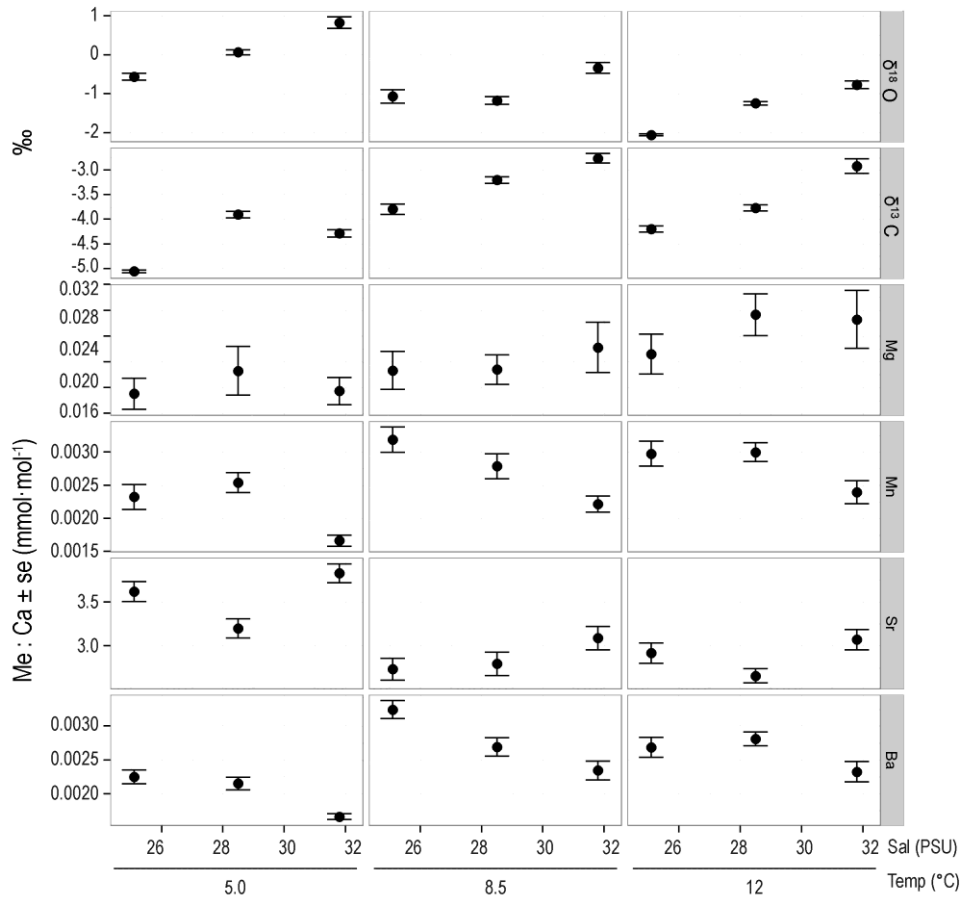


Figure 4: Mean Me:Ca_{Otolith} and isotopic ratios for temperature and salinity treatments (n=3 respectively) of juvenile *Gadus morhua* otoliths. Isotopes in ‰ relative to VPDB. Error bars represent ± 1 standard error. Figure 5: Mean partition coefficients (D_{Me}) for juvenile *Gadus morhua* otoliths reared under temperature and salinity experimental treatments). Mean D_{Mg} values are multiplied by 10^5 .

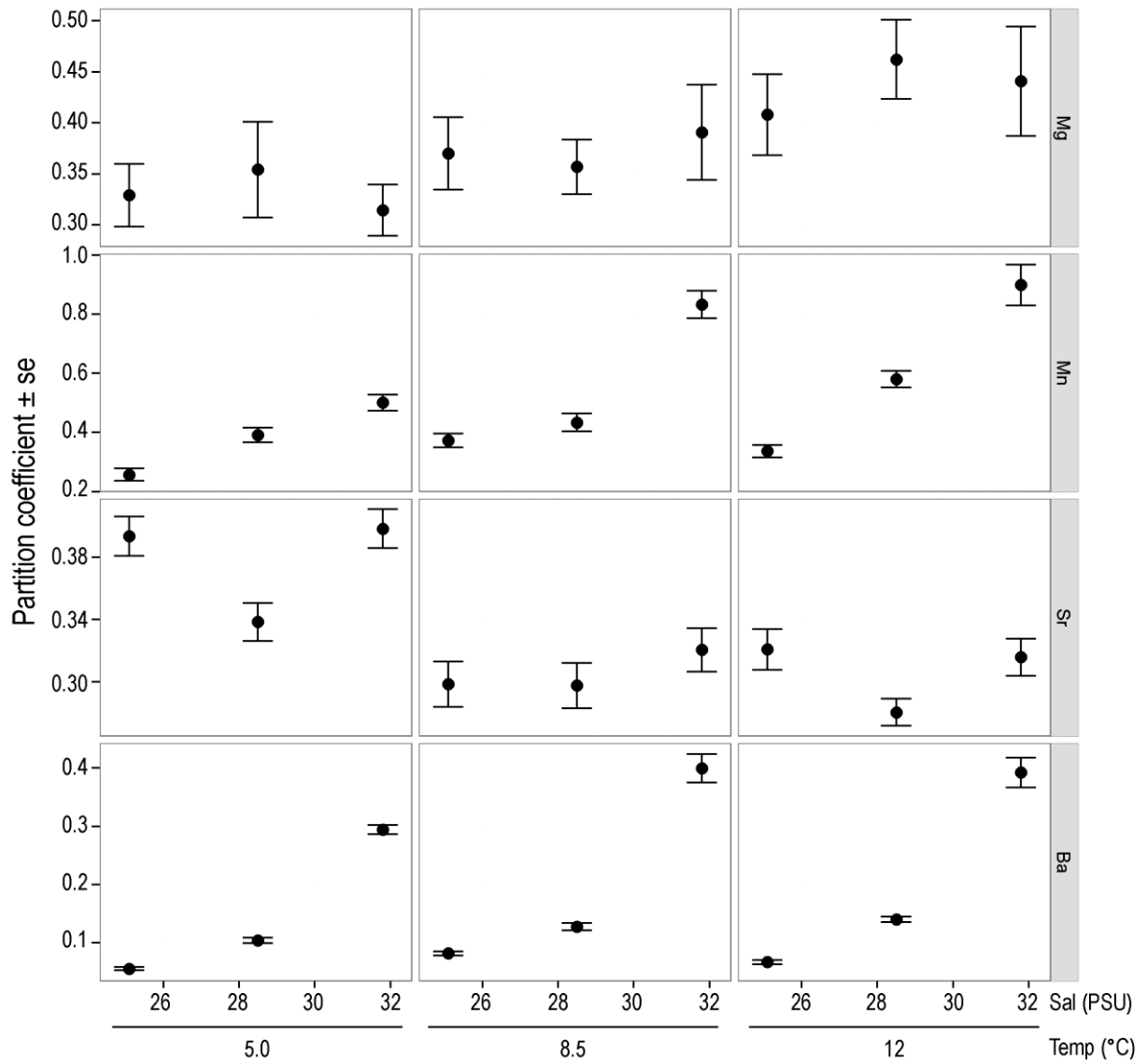


Figure 5. Mean partition coefficients (D_{me}) for juvenile *G. morhua* otoliths reared under temperature and salinity experimental treatments. Mean D_{mg} values are multiplied by 10^5 .

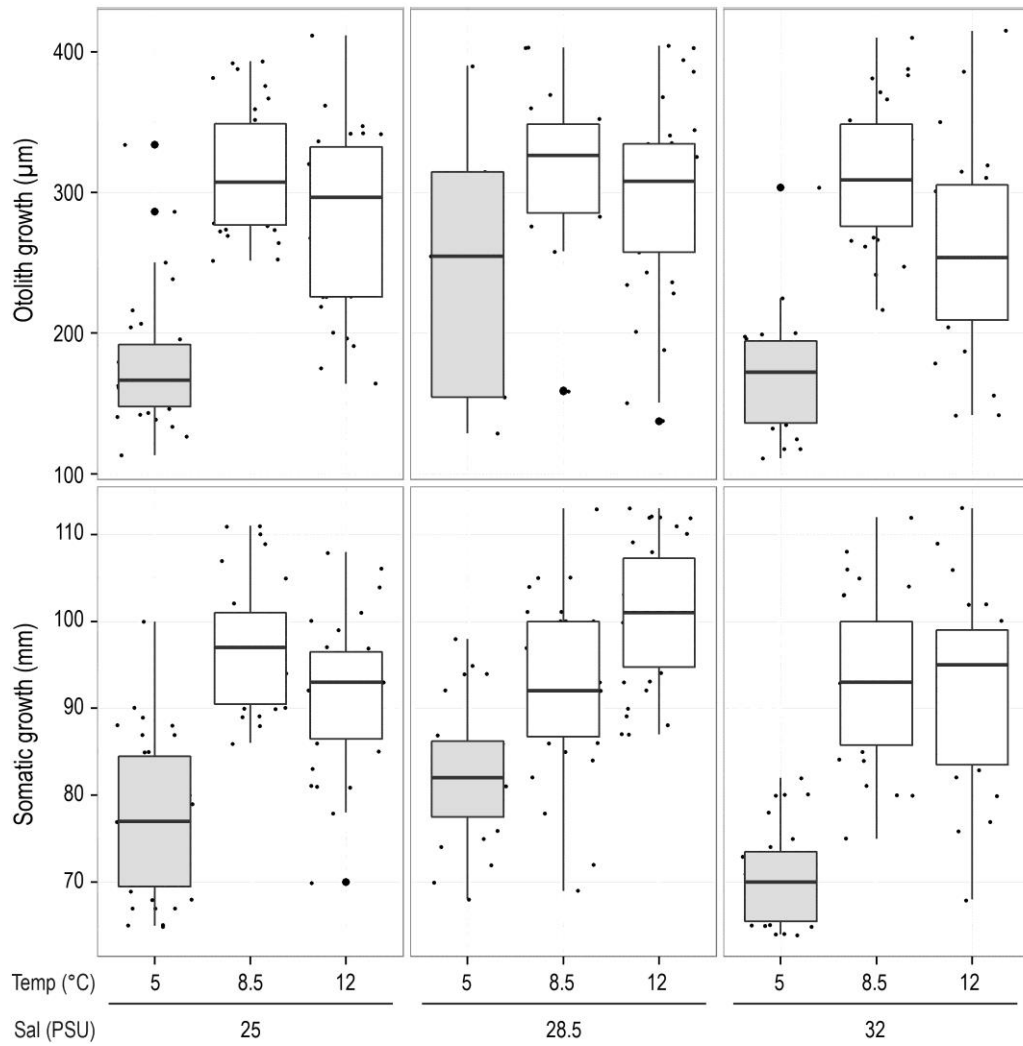


Figure 6. Summary of somatic and otolith growth throughout the experiment. Boxplot fill colours denote results of within treatment Tukey's post-hoc tests ($\alpha < 0.05$).

Supplementary material:

Table S1. Correlations between growth and elemental partition coefficients and isotope values in juvenile *Gadus morhua* grown under experimental conditions. Pearson correlation coefficients presented with *Benjamini-Hochberg* adjusted α controlling for multiple comparisons * $\alpha < 0.05$, ** $\alpha < 0.01$. Isotopes in ‰ relative to VPDB. Sample size of each treatment and element is denoted by n.

	Temp	Low			Med			High			Pooled
	Sal	Low	Med	High	Low	Med	High	Low	Med	High	
D _{Mg}	Somatic	0.15	0.03	-0.01	0.29	0.36	-0.27	0.1	0.05	-0.17	0.21*
	Otolith	0.17	-0.2	0.6	0.36	0.27	0.26	0.22	-0.17	-0.22	0.19*
	n	37	21	28	31	33	27	29	38	25	269
D _{Mn}	Somatic	0.26	-0.52	-0.19	0.41	0.42	0.12	0.06	0.14	0.02	0.33*
	Otolith	0.41	-0.17	0.28	0.45	0.66*	0.55*	0.36	0.16	0.05	0.34*
	n	39	21	25	28	31	29	31	39	24	267
D _{Sr}	Somatic	-0.32	0.87	-0.39	-0.3	-0.4	-0.51	-0.14	-0.01	-0.47	-0.56*
	Otolith	-0.42	-0.25	0.01	-0.48	-0.74*	-0.42	-0.56*	-0.28	-0.21	-0.57*
	n	37	22	24	27	32	29	29	38	27	265
D _{Ba}	Somatic	-0.05	-0.63	0.21	0.17	0.05	0.33	0.03	-0.04	-0.1	0.03
	Otolith	0.08	0.15	0.17	0.22	0.2	0.33	0.36	0.32	0.39	0.13
	n	33	23	28	27	32	29	31	39	27	269
$\delta^{13}\text{C}$	Somatic	0.19	-0.03	-0.42	0.08	-0.04	0.01	0.26	0.7	0.22	0.45*
	Otolith	0.31	0.07	-0.6	-0.07	-0.35	0.22	0.26	-0.5	0.01	0.37*
	n	8	15	16	13	15	15	11	10	14	116
$\delta^{18}\text{O}$	Somatic	-0.27	0.31	-0.24	0.38	-0.33	-0.06	-0.69	0.35	0.27	-0.41*
	Otolith	0.11	0.11	0	0.06	0.1	0.29	-0.86*	0.11	0.08	-0.34*
	n	14	14	16	11	13	15	9	10	14	116

References

- Barnes, T. C., and Gillanders, B. M. 2013. Combined effects of extrinsic and intrinsic factors on otolith chemistry: implications for environmental reconstructions. *Canadian Journal of Fisheries and Aquatic Sciences*, 70: 1159-1166.
- Bath, G. E., Thorrold, S. R., Jones, C. M., Campana, S. E., McLaren, J. W., and Lam, J. W. H. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica Et Cosmochimica Acta*, 64: 1705-1714.
- Beckman, D. W., and Schulz, R. G. 1996. A simple method for marking fish otoliths with alizarin compounds. *Transactions of the American Fisheries Society*, 125: 146-149.
- Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate - a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B-Methodological*, 57: 289-300.
- Bjornsson, B. 1993. Swimming speed and swimming metabolism of Atlantic cod (*Gadus morhua*) in relation to available food: a laboratory study. *Canadian Journal of Fisheries and Aquatic Sciences*, 50: 2542-2551.
- Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., and White, J. S. S. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution*, 24: 127-135.
- Bolland, K. 2008. Effects of temperature and salinity on growth of juvenile cod, *Gadus morhua*. In *Biology*, p. 40. Memorial University of Newfoundland, St. John's.
- Bradbury, I. R., Campana, S. E., and Bentzen, P. 2008. Otolith elemental composition and adult tagging reveal spawning site fidelity and estuarine dependency in rainbow smelt. *Marine Ecology Progress Series*, 368: 255-268.
- Bradbury, I. R., Snelgrove, P. V. R., and Fraser, S. 2000. Transport and development of eggs and larvae of Atlantic cod, *Gadus morhua*, in relation to spawning time and location in coastal Newfoundland. *Canadian Journal of Fisheries and Aquatic Sciences*, 57: 1761-1772.
- Brander, K. M. 1995. The effect of temperature on growth of Atlantic cod (*Gadus morhua* L.). *ICES Journal of Marine Science*, 52: 1-10.
- Brown, R. J., and Severin, K. P. 2009. Otolith chemistry analyses indicate that water Sr:Ca is the primary factor influencing otolith Sr:Ca for freshwater and diadromous fish but not for marine fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 66: 1790-1808.
- Buckel, J. A., Sharack, B. L., and Zdanowicz, V. S. 2004. Effect of diet on otolith composition in *Pomatomus saltatrix*, an estuarine piscivore. *Journal of Fish Biology*, 64: 1469-1484.
- Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. *Marine Ecology Progress Series*, 188: 263-297.
- Campana, S. E., Chouinard, G. A., Hanson, J. M., and Frechet, A. 1999. Mixing and migration of overwintering Atlantic cod (*Gadus morhua*) stocks near the mouth of the Gulf of St. Lawrence. *Canadian Journal of Fisheries and Aquatic Sciences*, 56: 1873-1881.
- Campana, S. E., Chouinard, G. A., Hanson, J. M., Frechet, A., and Bratney, J. 2000. Otolith elemental fingerprints as biological tracers of fish stocks. *Fisheries Research*, 46: 343-357.
- Collingsworth, P. D., Van Tassell, J. J., Olesik, J. W., and Marschall, E. A. 2010. Effects of temperature and elemental concentration on the chemical composition of juvenile yellow perch (*Perca flavescens*) otoliths. *Canadian Journal of Fisheries and Aquatic Sciences*, 67: 1187-1196.
- Craig, J. D. C., and Colbourne, E. B. 2004. Temperature Variability in the Coastal Waters of Eastern Newfoundland. Research Document: Canadian Science Advisory Secretariat, 2004/004.

- D'Avignon, G., and Rose, G. A. 2013. Otolith elemental fingerprints distinguish Atlantic cod spawning areas in Newfoundland and Labrador. *Fisheries Research*, 147: 1-9.
- Dalley, E. L., and Anderson, J. T. 1997. Age-dependent distribution of demersal juvenile Atlantic cod (*Gadus morhua*) in inshore/offshore northeast Newfoundland. *Canadian Journal of Fisheries and Aquatic Sciences*, 54: 168-176.
- de Vries, M. C., Gillanders, B. M., and Elsdon, T. S. 2005. Facilitation of barium uptake into fish otoliths: Influence of strontium concentration and salinity. *Geochimica Et Cosmochimica Acta*, 69: 4061-4072.
- DiMaria, R. A., Miller, J. A., and Hurst, T. P. 2010. Temperature and growth effects on otolith elemental chemistry of larval Pacific cod, *Gadus macrocephalus*. *Environmental Biology of Fishes*, 89: 453-462.
- Elsdon, T. S., and Gillanders, B. M. 2002. Interactive effects of temperature and salinity on otolith chemistry: challenges for determining environmental histories of fish. *Canadian Journal of Fisheries and Aquatic Sciences*, 59: 1796-1808.
- Elsdon, T. S., and Gillanders, B. M. 2003. Relationship between water and otolith elemental concentrations in juvenile black bream *Acanthopagrus butcheri*. *Marine Ecology Progress Series*, 260: 263-272.
- Elsdon, T. S., Wells, B. K., Campana, S. E., Gillanders, B. M., Jones, C. M., Limburg, K. E., Secor, D. H., et al. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. *In Oceanography and Marine Biology: An Annual Review*, Vol 46, pp. 297-+. Ed. by R. N. Gibson, R. J. A. Atkinson, and J. D. M. Gordon.
- Epstein, S., and Mayeda, T. 1953. Variation of O-18 content of waters from natural sources. *Geochimica Et Cosmochimica Acta*, 4: 213-224.
- Farmer, T. M., DeVries, D. R., Wright, R. A., and Gagnon, J. E. 2013. Using Seasonal Variation in Otolith Microchemical Composition to Indicate Largemouth Bass and Southern Flounder Residency Patterns across an Estuarine Salinity Gradient. *Transactions of the American Fisheries Society*, 142: 1415-1429.
- Farrell, J., and Campana, S. E. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comparative Biochemistry and Physiology a-Physiology*, 115: 103-109.
- Fowler, A. J., Campana, S. E., Jones, C. M., and Thorrold, S. R. 1995. Experimental assessment of the effect of temperature and salinity on elemental composition of otoliths using laser-ablation ICPMS. *Canadian Journal of Fisheries and Aquatic Sciences*, 52: 1431-1441.
- Gao, Y. W. 2002. Regime shift signatures from stable oxygen isotopic records of otoliths of Atlantic cod (*Gadus morhua*). *Isotopes in Environmental and Health Studies*, 38: 251-263.
- Gillanders, B. M. 2005. Using elemental chemistry of fish otoliths to determine connectivity between estuarine and coastal habitats. *Estuarine Coastal and Shelf Science*, 64: 47-57.
- Grossman, E. L., and Ku, T. L. 1986. Oxygen and carbon isotope fractionation in biogenic aragonite - temperature effects. *Chemical Geology*, 59: 59-74.
- Hoff, G. R., and Fuiman, L. A. 1995. Environmentally-induced variation in elemental composition of red drum (*Sciaenops ocellatus*) otoliths. *Bulletin of Marine Science*, 56: 578-591.
- Hoie, H., and Folkvord, A. 2006. Estimating the timing of growth rings in Atlantic cod otoliths using stable oxygen isotopes. *Journal of Fish Biology*, 68: 826-837.
- Hoie, H., Folkvord, A., and Otterlei, E. 2003. Effect of somatic and otolith growth rate on stable isotopic composition of early juvenile cod (*Gadus morhua* L.) otoliths. *Journal of Experimental Marine Biology and Ecology*, 289: 41-58.

- Hoie, H., Otterlei, E., and Folkvord, A. 2004. Temperature-dependent fractionation of stable oxygen isotopes in otoliths of juvenile cod (*Gadus morhua* L.). *Ices Journal of Marine Science*, 61: 243-251.
- Jamieson, R. E., Schwarcz, H. P., and Bratney, J. 2004. Carbon isotopic records from the otoliths of Atlantic cod (*Gadus morhua*) from eastern Newfoundland, Canada. *Fisheries Research*, 68: 83-97.
- Kalish, J. M. 1991. Determinants of otolith chemistry: seasonal variation in the composition of blood plasma, endolymph and otoliths of bearded rock cod *Pseudophycis barbatus*. *Marine Ecology Progress Series*, 74: 137-159.
- Laurel, B. J., Gregory, R. S., and Brown, J. A. 2003. Settlement and distribution of age-0 juvenile cod, *Gadus morhua* and *G. ogac*, following a large-scale habitat manipulation. *Marine Ecology Progress Series*, 262: 241-252.
- Limburg, K. E., Olson, C., Walther, Y., Dale, D., Slomp, C. P., and Hoie, H. 2011. Tracking Baltic hypoxia and cod migration over millennia with natural tags. *Proceedings of the National Academy of Sciences of the United States of America*, 108: E177-E182.
- Limburg, K. E., Walther, B. D., Lu, Z., Jackman, G., Mohan, J., Walther, Y., Nissling, A., et al. 2014. In search of the dead zone: Use of otoliths for tracking fish exposure to hypoxia. *Journal of Marine Systems*, 141: 167-178.
- Martin, G. B., and Thorrold, S. R. 2005. Temperature and salinity effects on magnesium, manganese, and barium incorporation in otoliths of larval and early juvenile spot *Leiostomus xanthurus*. *Marine Ecology Progress Series*, 293: 223-232.
- Martin, G. B., Thorrold, S. R., and Jones, C. M. 2004. Temperature and salinity effects on strontium incorporation in otoliths of larval spot (*Leiostomus xanthurus*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61: 34-42.
- Martin, G. B., and Wuenschel, M. J. 2006. Effect of temperature and salinity on otolith element incorporation in juvenile gray snapper *Lutjanus griseus*. *Marine Ecology Progress Series*, 324: 229-239.
- McConnaughey, T. A., Burdett, J., Whelan, J. F., and Paull, C. K. 1997. Carbon isotopes in biological carbonates: Respiration and photosynthesis. *Geochimica Et Cosmochimica Acta*, 61: 611-622.
- Melancon, S., Fryer, B. J., and Markham, J. L. 2009. Chemical analysis of endolymph and the growing otolith: fractionation of metals in freshwater fish species. *Environmental Toxicology and Chemistry*, 28: 1279-1287.
- Miller, J. A. 2009. The effects of temperature and water concentration on the otolith incorporation of barium and manganese in black rockfish *Sebastes melanops*. *Journal of Fish Biology*, 75: 39-60.
- Miller, J. A. 2011. Effects of water temperature and barium concentration on otolith composition along a salinity gradient: Implications for migratory reconstructions. *Journal of Experimental Marine Biology and Ecology*, 405: 42-52.
- Milton, D. A., and Chenery, S. R. 2001. Sources and uptake of trace metals in otoliths of juvenile barramundi (*Lates calcarifer*). *Journal of Experimental Marine Biology and Ecology*, 264: 47-65.
- Morse, J. W., and Bender, M. L. 1990. Partition-coefficients in calcite: examination of factors influencing the validity of experimental results and their application to natural systems. *Chemical Geology*, 82: 265-277.
- Ostermann, D. R., and Curry, W. B. 2000. Calibration of stable isotopic data: An enriched delta O-18 standard used for source gas mixing detection and correction. *Paleoceanography*, 15: 353-360.

- Otterlei, E., Folkvord, A., and Nyhammer, G. 2002. Temperature dependent otolith growth of larval and early juvenile Atlantic cod (*Gadus morhua*). *Ices Journal of Marine Science*, 59: 850-860.
- R Development Core Team 2014. R: A language and environment for statistical computing. *In* Vienna Austria: R Foundation for Statistical Computing.
- Reis-Santos, P., Tanner, S. E., Elsdon, T. S., Cabral, H. N., and Gillanders, B. M. 2013. Effects of temperature, salinity and water composition on otolith elemental incorporation of *Dicentrarchus labrax*. *Journal of Experimental Marine Biology and Ecology*, 446: 245-252.
- Reis-Santos, P., Vasconcelos, R. P., Ruano, M., Latkoczy, C., Gunther, D., Costa, M. J., and Cabral, H. 2008. Interspecific variations of otolith chemistry in estuarine fish nurseries. *Journal of Fish Biology*, 72: 2595-2614.
- Rose, G. A., Nelson, R. J., and Mello, L. G. S. 2011. Isolation or metapopulation: whence and whither the Smith Sound cod? *Canadian Journal of Fisheries and Aquatic Sciences*, 68: 152-169.
- Ruzzante, D. E., Wroblewski, J. S., Taggart, C. T., Smedbol, R. K., Cook, D., and Goddard, S. V. 2000. Bay-scale population structure in coastal Atlantic cod in Labrador and Newfoundland, Canada. *Journal of Fish Biology*, 56: 431-447.
- Schwarcz, H. P., Gao, Y., Campana, S., Browne, D., Knyf, M., and Brand, U. 1998. Stable carbon isotope variations in otoliths of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences*, 55: 1798-1806.
- Secor, D. H., Hendersonarzapalo, A., and Piccoli, P. M. 1995. Can otolith microchemistry chart patterns of migration and habitat utilization in anadromous fishes? *Journal of Experimental Marine Biology and Ecology*, 192: 15-33.
- Stanley, R. R. E., deyoung, B., Snelgrove, P. V. R., and Gregory, R. S. 2013. Factors Regulating Early Life History Dispersal of Atlantic Cod (*Gadus morhua*) from Coastal Newfoundland. *PLoS ONE*, 8.
- Sturgeon, R. E., Willie, S. N., Yang, L., Greenberg, R., Spatz, R. O., Chen, Z., Sriver, C., et al. 2005. Certification of a fish otolith reference material in support of quality assurance for trace element analysis. *Journal of Analytical Atomic Spectrometry*, 20: 1067-1071.
- Thorisson, K., Jonsdottir, I. G., Marteinsdottir, G., and Campana, S. E. 2011. The use of otolith chemistry to determine the juvenile source of spawning cod in Icelandic waters. *Ices Journal of Marine Science*, 68: 98-106.
- Thorrold, S., and Swearer, S. E. 2009. Otolith chemistry. *In* *Tropical fish otoliths: Information for assessment, management and ecology*, pp. 249-296. Ed. by B. S. Green, G. A. Begg, G. Carlos, and B. Mapstone. Springer, Netherlands.
- Thorrold, S. R., Campana, S. E., Jones, C. M., and Swart, P. K. 1997a. Factors determining delta C-13 and delta O-18 fractionation in aragonitic otoliths of marine fish. *Geochimica Et Cosmochimica Acta*, 61: 2909-2919.
- Thorrold, S. R., Jones, C. M., and Campana, S. E. 1997b. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). *Limnology and Oceanography*, 42: 102-111.
- Thorrold, S. R., Jones, C. M., Campana, S. E., McLaren, J. W., and Lam, J. W. H. 1998. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). *Limnology and Oceanography*, 43: 1826-1835.
- Thorrold, S. R., Latkoczy, C., Swart, P. K., and Jones, C. M. 2001. Natal homing in a marine fish metapopulation. *Science*, 291: 297-299.
- Townsend, D. W., Radtke, R. L., Malone, D. P., and Wallinga, J. P. 1995. Use of otolith strontium - calcium ratios for hindcasting larval cod *Gadus morhua* distributions relative to water masses on Georges Bank. *Marine Ecology Progress Series*, 119: 37-44.

- Walther, B. D., and Thorrold, S. R. 2006. Water, not food, contributes the majority of strontium and barium deposited in the otoliths of a marine fish. *Marine Ecology Progress Series*, 311: 125-130.
- Webb, S. D., Woodcock, S. H., and Gillanders, B. M. 2012. Sources of otolith barium and strontium in estuarine fish and the influence of salinity and temperature. *Marine Ecology Progress Series*, 453: 189-199.
- Weidman, C. R., and Millner, R. 2000. High-resolution stable isotope records from North Atlantic cod. *Fisheries Research*, 46: 327-342.
- Woodcock, S. H., Munro, A. R., Crook, D. A., and Gillanders, B. M. 2012. Incorporation of magnesium into fish otoliths: Determining contribution from water and diet. *Geochimica Et Cosmochimica Acta*, 94: 12-21.
- Worthington, D. G., Doherty, P. J., and Fowler, A. J. 1995. Variation in the relationship between otolith weight and age - implications for the estimation of age of 2 tropical damselfish (*Pomacentrus moluccensis* and *P. wardi*). *Canadian Journal of Fisheries and Aquatic Sciences*, 52: 233-242.
- Yoshinaga, J., Nakama, A., Morita, M., and Edmonds, J. S. 2000. Fish otolith reference material for quality assurance of chemical analyses. *Marine Chemistry*, 69: 91-97.